CERTIFICATE OF FACSIMILE TRANSMISSION

I hereby certify that this correspondence is being transmitted to the U.S. Patent & Trademark Office in accordance with 37 CFR § 1.6(d) on the date indicated.

Karen Zielen (Name) — Dec. 24 2004 (Date)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventors: Cech et al.

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Serial No: 09/432,503

Docket: 015389-002611US; 018/063c

Title: INCREASING THE PROLIFERATIVE CAPACITY OF CELLS USING TELOMERASE REVERSE TRANSCRIPTASE

Art Unit: 1645

Examiner: J. Eric Angell, Ph.D.

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DECLARATION UNDER 37 CFR § 1.132
EDWARD D. WIRTH III, M.D., Ph.D.

Commissioner for Patents
Alexandria VA 22313

Dear Sir:

I, EDWARD WIRTH, do hereby declare as follows:

I am the Associate Medical Director of Geron Corporation, a co-owner of this invention. A copy of my *curriculum vitae* accompanies this Declaration. While at the University of Florida, I was Principal Investigator for an investigator-sponsored Phase I clinical trial to test treatment of Syringomyelia. I am now designing a clinical trial for the treatment of acute spinal cord injury using oligodendrocytes derived from human embryonic stem cells.

I have been asked by the owners of this invention to address an issue raised during consideration of this patent application by the U.S. Patent & Trademark Office.

The application describes the isolation and characterization of the human gene for telomerase reverse transcriptase (TRT), and its use for various purposes of commercial and clinical importance. One example is the use of TRT to extend the proliferative capacity of cells. The telomerase enzyme is made up of the catalytic protein, TRT, associated with telomerase RNA component. Most mature human cells including hepatocytes have virtually no TRT expression. Thus, expressing TRT in a cell reconstitutes telomerase activity, restores telomere length, and increases the cell's proliferative capacity.

The effectiveness of TRT gene therapy to increase proliferative capacity in vitro has been confirmed extensively (e.g., Bodnar et al., Science 279:334, 1998; Vaziri et al., Curr Biol. 8:279, 1998; Steinert et al., Biochem Biophys Res Commun. 273:1095, 2000; US 2003/0175967 A1). TRT gene therapy has also been shown to increase the rate of wound healing in vivo, which is attributed to increased proliferative capacity of skin keratinocytes (WO 02/091999).

I understand the Office has questioned whether TRT would increase the proliferative capacity of cells when used as gene therapy in vivo. The Office has previously been provided with a copy of the article by Rudolph et al., entitled "Inhibition of Experimental Liver Cirrhosis in Mice by Telomerase Gene Delivery" (Science 287:1253-1257, 2000), but questions whether the experiments described by Rudolph et al. provide a good model for TRT therapy in humans.

The value of the model comes from the pattern of expression for TRT and the RNA component in humans and in mice. Normally, expression of TRT is limiting in mature human cells, while both TRT and RNA component are expressed in mouse cells. The following table provides an overview:

Expression of Telomerase *in vivo*

	TRT (protein component)	RNA component
adult human hepatocytes	virtually no expression	constitutive expression
normal mouse hepatocytes	constitutive expression	constitutive expression
hepatocytes from mTR knockout mice (Rudolph et al.)	constitutive expression	no expression

Thus, transfection with the gene for the RNA component in Rudolph's experiments supplied the missing component of telomerase in mice, just like supplying TRT to the liver of humans would provide the component of telomerase missing from human cells. Both situations allow the cells to make the whole telomerase enzyme, which can then extend telomere length, thereby increasing proliferative capacity of the cells.

In the introductory paragraphs of the article, Rudolph et al. review the known pathology in liver cirrhosis. Hepatocyte destruction and fibrosis increases the rate of hepatocyte turnover, but the pathological process culminates in fatal end-stage liver failure, marked by extensive fibrotic replacement and cessation of hepatocyte proliferation (S.L. Friedman, N. Engl. J. Med. 328:1828, 1993; Williams et al., Postgrad Med. J. 74:193, 1998; Alcolado et al., Clin. Sci. (Lond.) 92:103, 1997; Delhaye et al., Hepatology 23:1003, 1996). Sustained hepatocyte turnover accelerates the pace of telomere attrition in the human cirrhotic liver (Kitada et al., Biochem. Biophys Res. Commun. 211:33, 1995; Miura et al., Cancer Genet. Cytogenet. 93:56, 1997; Liver 16:293, 1996).

Figure 2A shows that cells in mTR knockout mice have partly shortened telomeres after two generations of in-breading, and by the sixth generation (G6), telomere length has deceased to about 50% of normal ($p=0.047$).

Figure 2B shows that G2/M cell cycle block (a 2- to 3-fold increase in the G2/M fraction) in regenerating hepatocytes of G6 knockout mice after partial hepatectomy. This indicates that the cells are attempting to enter a replicative phase, but are no longer successful in doing so. The impaired cell progression through mitosis delays restoration of the liver mass.

Figure 3C shows that telomere shortening accelerates the development of cirrhosis in response to liver damage due to CCl_4. Marked steatosis and fibrosis are evident in livers of G6 knockout mice, but not normal mice.

Figure 4B shows that adenovirus mediated therapy using the mouse telomerase RNA component (mTR) restores telomerase enzyme activity (measured by TRAP assay) in the liver of the knockout mice 48 hours after transfection.

Figure 5A shows that reactivation of telomerase activity by mTR gene therapy increases the proliferative capacity of hepatocytes in the G6 knockouts. The vector causes expression of both mTR and green fluorescence protein (GFP), so that the proliferation of transfected cells can be followed in situ. In the control animals, the transfected cells were lost 2 weeks after infection, apparently because there was enough reserve replicative capacity in the other cells. However, in the G6 knockout animals, about 50% of the hepatocytes contained the mTR-GFP vector ($p=0.014$). In contrast, there were few surviving cells in G6 knockout mice treated with a vector containing GFP alone ($p=0.009$).

This shows that transfecting cells having low replicative capacity in vivo to induce mTR expression allows the labeled transfected cells to outgrow the rest of the population — attributable to a restoration of proliferative capacity in the successfully transfected cells.

Figure 5B shows that transfection with the mTR-GFP vector also made animals resistant to cirrhosis induced by four weeks of CCl_4 administration, as measured by albumin synthesis and ascites formation. Thus, restoring replicative capacity to the liver cells has significant therapeutic benefit in this model.

The model used in the Rudolph article parallels the situation in human chronic liver disease. Liver failure resulting from cirrhosis in human patients is attributable to severely limited proliferative capacity of the hepatocytes in the affected liver. Using an adenovirus vector to deliver the TRT gene to the liver in humans would cause TRT to be expressed in hepatocytes, just as delivering the mTR gene to the liver of G6 knockout mice caused mTR to be expressed in the liver.

Inducing TRT expression in human hepatocytes in vivo would renew expression of the missing component of the telomerase enzyme, which in turn would restore telomerase enzyme activity and telomere length, thus causing increased proliferative capacity. In parallel to the observations of Rudolph et al., the increasing proliferative capacity of hepatocytes in the liver of cirrhotic patients would limit further damage and allow the liver cells to regrow.

I hereby declare that all statements made in this Declaration of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

12/22/04
Date

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